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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C07K 5/06, 5/02, A61K 37/64 — A1

(11) International Publication Number: WO 94/29335

(43) International Publication Date: 22 December 1994 (22.12.94)

(21) International Application Number: PCT/SE94/00534

(22) International Filing Date: 2 June 1994 (02.06.94)

(30) Priority Data:
9301911-5 3 June 1993 (03.06.93) SE

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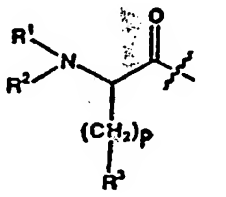
(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: NEW PEPTIDES DERIVATIVES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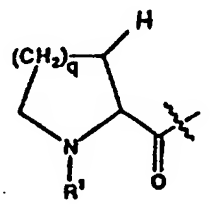
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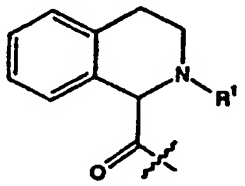
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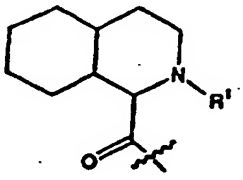
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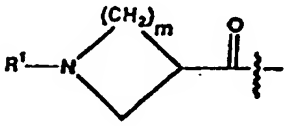
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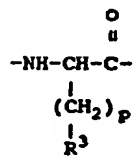
(a)

(57) Abstract

A compound of the general formula (I): A^1 - A^2 -NH-$(CH_2)_n$ - NH-C(NH)-NH_2, wherein n is an integer 2, 3, 4, 5, or 6; preferably 3 or 4; A^1 represents a structural fragment of formulae (IIa), (IIb), (IIc), (IId) or (IIe); A^2 represents a structural fragment (a), as well as processes for the preparation thereof, the use and the pharmaceutical formulations.

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New peptide derivatives

DESCRIPTION

In its broad sense this invention relates to protease inhibition and treatment of inflammatory diseaes. More specifically this invention relates to new competitive inhibitors of trypsin-like serine proteases such as kininogenases, their synthesis, pharmaceutical compositions containing the compounds as active ingredients, and the use of the compounds for treatment of inflammatory disorders, e.g. asthma, rhinitis, urticaria, inflammatory bowel diseaes, and arthritis.

BACKGROUND

Kininogenases are serine proteases that act on kininogens to produce kinins (bradykinin, kallidin, and Met-Lys-bradykinin). Plasma kallikrein, tissue kallikrein, and mast cell tryptase represent important kininogenases.

Kinins (bradykinin, kallidin) are generally involved in inflammation. For example, the active inflammation process is associated with increased permeability of the blood vessels resulting in extravasation of plasma into the tissue. The ensuing plasma exudate contains all the protein systems of circulating blood. The plasma-derived kininogens inevitably will be interacting with different kallikreins, forming kinins continually as long as the active plasma exudation process is ongoing. Plasma exudation occurs independent of the mechanisms that are involved in the inflammation, whether it is allergy, infection or other factors (Persson et al., Editorial, Thorax, 1992, 47:993-1000). Plasma exudation is thus a feature of many diseases including asthma, rhinitis, common cold, and inflammatory bowel diseases. Particulary in allergy mast cell tryptase will be released (Salomonsson et al., Am. Rev. Respir. Dis., 1992, 146:1535-1542) to

contribute to kinin formation and other pathogenic events in asthma, rhinitis, and intestinal diseases.

The kinins are biologically highly active substances with smooth muscle effects, sectretory effects, neurogenic effects, and actions that may perpetuate inflammatory processes including activation of phospholipase A^2 and increasing vascular permeability. The latter action potentially induces a vicious circle with kinins providing for the generation of more kinins etc.

Tissue kallikrein cleaves primarily low molecular weight kininogen to produce kallidin and plasma kallikrein preferably releases bradykinin from high molecular weight kininogen.

PRIOR ART

Inhibitors of kallikrein based on the amino acid sequence around the cleavage site (-Ser-Pro-Phe-Arg — Ser-Ser-Arg-) have been reported earlier.

The arginine chloromethyl ketones were reported as plasma kallikrein inhibitors by Kettner and Shaw in Biochemistry 1978, 17:4778-4784 and Meth. Enzym. 1981, 80:826-842.

Likewise, esters and amides were reported by Fareed et al. in Ann. N.Y. Acad. Sci. 1981, 370:765-784 to be plasma kallikrein inhibitors.

In EP-A2-0,195,212 protease enzym inhibitors, based on analogues of peptidase substrates, including kallikrein, are described.

Inhibitors of trypsin like serine proteases, such as thrombin and kallikrein, based on C-terminal boronic acid derivatives

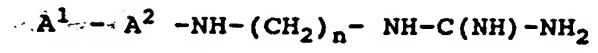
of arginine and isothiouronium analogues thereof have been reported in EP-A2-0,293,881.

In WO 92/04371 a series of kallikrein inhibitors with carbonyl-activating or binding groups are described.

DISCLOSURE OF THE INVENTION

An objective of the present invention is to provide novel and potent kallikrein inhibitors with competitive inhibitory activity towards the enzyme i.e. causing reversible inhibition. A further objective is to obtain inhibitors which can be given orally, dermally, rectally, or via the inhalation route.

According to the invention it has been found that compounds of the general Formula I, either as such or in the form of physiologically acceptable salts, and including stereoisomers, are potent inhibitors of serine proteases and especially kallikreins:

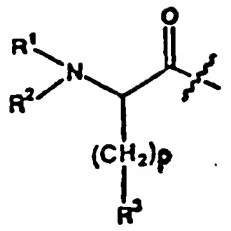


Formula I

wherein:

n is an integer 2, 3, 4, 5, or 6; preferably 3 or 4;

A^1 represents a structural fragment of Formulae IIa, IIb, IIc, IId or IIe;



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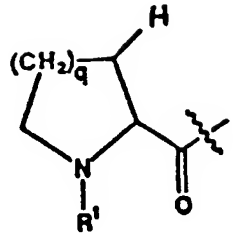

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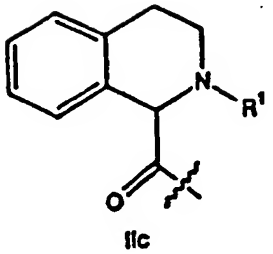

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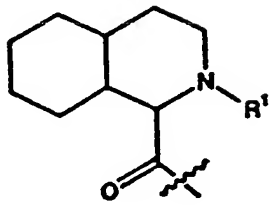

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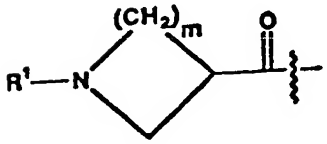


IIe

wherein:

p is an integer 0,1 or 2;

m is an integer 1, 2, 3, or 4, preferably 2;

q is an integer 0,1 or 2, preferably 1;

R^1 represents H, an alkyl group having 1 to 4 carbon atoms, a hydroxyalkyl group having 2-3 carbon atoms or $R^{11}OOC$-alkyl-, where the alkyl group has 1 to 4 carbon atoms and R^{11} is H or an alkyl group having 1 to 4 carbon atoms, or

R^1 represents $R^{12}OOC$-1,4-phenyl-CH_2-, wherein R^{12} is H or an alkyl group having 1 to 4 carbon atoms, or

R^1 represents R^{13}-NH-CO-alkyl-, wherein the alkyl group has 1 to 4 carbon atoms and is possibly substituted alpha to the carbonyl with an alkyl group having 1 to 4 carbon atoms and wherein R^{13} is H or an alkyl group having 1 to 4 carbon atoms or $-CH_2COOR^{12}$, wherein R^{12} is as defined above, or

R^1 represents $R^{14}SO_2$-, Ph(4-$COOR^{12}$)-SO_2-, Ph(3-$COOR^{12}$)-SO_2-, or Ph(2-$COOR^{12}$)-SO_2-, wherein R^{12} is as defined above and R^{14} is an alkylgroup having 1-4 carbon atoms, or

R^1 represents CO-R^{15}, wherein R^{15} is an alkyl group having 1-4 carbon atoms, or

R^1 represents CO-OR^{15}, wherein R^{15} is as defined above, or

R^1 represent CO-$(CH_2)_p$-$COOR^{12}$, wherein R^{12} and p are as defined above, or

R^1 represents -$CH_2PO(OR^{16})_2$, wherein R^{16} is, individually at each occurrence, H, methyl or ethyl;

R^2 represents H or an alkyl group having 1 to 4 carbon atoms or $R^{21}OOC$-alkyl-, wherein the alkyl group has 1 to 4 carbon atoms and is possibly substituted in the position which is alpha to the carbonyl group, and the alpha substituent is a group R^{22}-$(CH_2)_p$-, wherein p is as defined above and R^{22} is methyl, phenyl, OH, $COOR^{21}$, and R^{21} is H or an alkyl group having 1 to 4 carbon atoms;

R^3 represents an alkyl group having 1-4 carbon atoms, or

R^3 represents a cyclohexyl- or cyclopentyl group, or

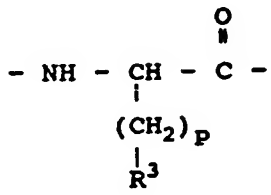
R^3 represents a phenyl group which may or may not be substituted with an alkyl group having 1 to 4 carbon atoms, or with a group OR^{21}, wherein R^{21} is as defined above or

R^3 represents a 1-napthyl, 2-naphtyl, 4-pyridyl, 3-pyrrolidyl, or a 3-indolyl group which may or may not be substituted with OR^{21} wherin R^{21} is as defined above and with p = 1; or

R^3 represent a cis- or trans-decalin group with p = 1; or

R^3 represents $Si(Me)_3$ or $CH(R^{31})_2$, wherein R^{31} is a cyclohexyl- or phenyl group;

A^2 represents a structural fragment



wherein R^3 and p are as defined above;

An alkyl group may be straight or branched unless specified otherwise. Alkyl groups having 1 to 4 carbon atoms are methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, s-butyl and t-butyl. When unsaturation is referred to, a carbon-carbon double bond is intended. Abbreviations are listed at the end of this specification.

According to the invention it has been found that compounds of the general Formula I, either as such or in the form of physiologically acceptable salts, and including stereoisomers, are potent inhibitors of trypsin-like serine proteases and especially plasma and/or tissue kallikrein: Compounds of Formula I having S-configuration on the A^2 amino acid are preferred ones, of those compounds also having R-configuration on the A^1 amino acid are particularly preferred ones.

Preferred compounds of the invention include:

H-(R)Cha-Phe-Agm
HOOC-CH_2-(R)Cha-Phe-Agm
H-(R)Cha-Phe-Nag

HOOC-CH_2-(R)Cha-Phe-Nag
CH_3-CO-(R)-Cha-Phe-Nag
CH_3-CH_2-(R)-Cha-Phe-Nag
HOOC-CO-(R)-Cha-Phe-Nag
HOOC-CH_2-(R)Phe-Phe-Agm
HOOC-CH_2-(R)Phe-Cha-Agm
HOOC-CH_2-(R)Cha-Cha-Agm
HOOC-CH_2-(R)Phe-Phe-Nag
HOOC-CH_2-(R)Phe-Cha-Nag
HOOC-CH_2-(R)Cha-Cha-Nag
HOOC-CH_2-(R)Cha-αNal-Agm
HOOC-CH_2-(R)Cha-βNal-Agm
H-(R)Phe-Cha-Agm
H-(R)Phe-Cha-Nag
H-(R)Phe-Phe-Agm
H-(R)Phe-Phe-Nag
CH_3-(R)Phe-Phe-Agm
CH_3-(R)Cha-Phe-Agm
CH_3-(R)Phe-Cha-Agm
HOOC-CH_2-(R)Pro-Phe-Agm
HOOC-CH_2-(R)Pro-Phe-Nag
H-(R)Pro-Phe-Agm
H-(R)Pro-Phe-Nag
CH_3-(R)Pro-Phe-Agm
CH_3-(R)Pro-Phe-Nag

Particularly preferred compounds are:

H-(R)Cha-Phe-Agm
HOOC-CH_2-(R)Cha-Phe-Agm
H-(R)Cha-Phe-Nag
HOOC-CH_2-(R)Cha-Phe-Nag
CH_3-CO-(R)-Cha-Phe-Nag
CH_3-CH_2-(R)-Cha-Phe-Nag
HOOC-CO-(R)-Cha-Phe-Nag

The best mode according to the invention known at present is to use the compound according to Example 4 namely.

$HOOC-CH_2-(R)Cha-Phe-Nag$

Medical and pharmaceutical use

The invention also provides compositions and methods for the treatment of physiological disorders and especially inflammatory diseases such as asthma, rhinitis, pancreatitis, uticaria, inflammatory bowel diseaes, and arthritis. An effective amount of Formula I with or without a physiologically acceptable carrier or diluent can be used solely or in combination with other therapeutic agents. Depending upon the disorder and patient to be treated the compositions may be administered via oral, dermal, nasal, tracheal, bronchial, parenteral, or rectal routes at varying doses.

The compounds inhibit the activity of kallikreins assessed with chromogenic substrates according to known procedures. The anti-inflammatory actions of the present compounds can for example be studied by their inhibition of allergen-induced exudative inflammatory processes in airway mucosa or gut mucosa.

Determinaton of the inhibition constant K_i for plasma kallikrein.

K_i determinations were made with a chromogenic substrate method, and performed on a Cobas Bio centrifugal analyzer manufactured by Roche (Basel, Switzerland). Residual enzyme activity after incubation of human plasma kallikrein with various concentrations of test compound was determined at three different substrate concentrations, and measured as change in optical absorbance at 405 nm and 37°C.

Human plasma kallikrein (E.C.3.4.21.34, Chromogenix AB, Mölndal, Sweden), 250 µl of 0.4 nkat/ml in buffer (0.05 mol/l Tris-HCl, pH 7.4, I 0.15 adjusted with NaCl) with bovine albumin 5 g/l (cat no 810033, ICI Biochemicals Ltd, High Wycombe, Bucks, GB), was incubated for 300 s with 80 µl of test compound solution in 0.15 mol/l NaCl containing albumin 10 g/l. An additional 10 µl of water was supplied in this step. Then 40 µl of kallikrein substrate (S-2302, Chromogenix AB, 1.25, 2.0 or 4.0 mmol/l in water) was added together with another 20 µl of water, and the absorbance change monitored.

K_i was evaluated from Dixon plots, i.e. diagrams of inhibitor concentration versus 1/ (ΔA/min), where the data for the different substrate concentrations form straight lines which intercept at $x = -K_i$.

Pharmaceutical preparations

The compounds of the Formula I will normally be administered by the oral, rectal, dermal, nasal or parenteral route in the form of pharmaceutical preparations comprising the active ingredient either as a free base or a pharmaceutical acceptable non-toxic organic or inorganic acid addition salt, e.g. the hydrochloride, hydrobromide, lactate, acetate, citrate and trifluoroacetate and the like in a pharmaceutically acceptable dosage form.

The dosage form may be a solid, semisolid or liquid preparation prepared by *per se* known techniques. Usually the active substance will constitute between 0.1 and 99 % by weight of the preparation, more specifically between 0.1 and 50 % by weight for preparations intended for parenteral administration and between 0.2 and 75 % by weight for preparations suitable for oral administration.

Suitable daily doses of the compounds of the invention in therapeutical treatment of humans are about 0.001-100 mg/kg

body weight at peroral administration and 0.001-50 mg/kg body weight at parenteral administration.

Preparation

A further objective of the invention is the mode of preparation of the compounds. The compounds of Formula I may be prepared by coupling of an N-terminally protected dipeptide (W_1-A^1-A^2-OH) or amino acid (W_1-A^2-OH), when a N-terminally protected amino acid is used a second amino acid is added afterwards using standard methods, to a compound

$$H_2N-(CH_2)_n-X$$

wherein A^1, A^2 and n are as defined with Formula I and X is an unprotected or protected guanidino group or a protected amino group, or a group transferable into an amino group, where the amino group is subsequently transferred into an unprotected or protected guanidino group, followed by removal of the protecting group(s) or deprotecting of the N-terminal nitrogen followed by alkylation of the N-terminal nitrogen and deprotection by known methods.

The coupling is accordingly done by one of the following methods:

Method I

Coupling of an N-terminally protected dipeptide, prepared by standard peptide coupling, with either a protected- or unprotected amino guanidine or a straight chain alkylamine carrying a protected or masked amino group at the terminal end of the alkyl chain, using standard peptide coupling, shown in the formula

$$W_1-A^1-A^2-OH \xrightarrow{H_2N-(CH_2)_n-X} W_1-A^1-A^2-NH(CH_2)_n-X$$

wherein A^1, A^2 and n are as defined in Formula I , W_1 is a N-teminal amino protecting group such as tert-butyloxy carbonyl and benzyloxy carbonyl and X is $-NH-C(NH)-NH_2$, $-NH-C(NH)-NH-W_2$, $-N(W_2)-C(NH)-NH-W_2$, $-NH-C(NW_2)-NH-W_2$ or $-NH-W_2$, where W_2 is an amine protecting group such as tert-butyloxy carbonyl or benzyloxy carbonyl, or X is a masked amino group such as azide, giving the protected peptide. The final compounds can be made in any of the following ways, depending on the nature of the X- group used: Removal of the protecting group(s) (when X= $-NH-C(NH)-NH_2$, $-N(W_2)-C(NH)-NH-W_2$, $-NH-C(NW_2)-NH-W_2$ or $-NH-C(NH)-NH-W_2$), or a selective deprotection of the W_1-group (e.g when X= $-NH-C(NH)-NH-W_2$, $-N(W_2)-C(NH)-NH-W_2$, $-NH-C(NW_2)-NH-W_2$, W_2 in this case must be orthogonal to W_1) followed by alkylation of the N-terminal nitrogen and deprotection or a selective deprotection/ unmasking of the terminal alkylamino function (X= $NH-W_2$, W_2 in this case must be orthogonal to W_1 or X= a masked aminogroup, such as azide) followed by a guanidation reaction, using standard methods, of the free amine and deprotection of the W_1-group.

Method II

Coupling of an N-terminally protected amino acid, prepared by standard methods, with either a protected- or unprotected amino guanidine or a straight chain alkylamine carrying a protected or masked amino group at the terminal end of the alkyl chain, using standard peptide coupling, shown in the formula

$$W_1-A^2-OH \quad + \quad H_2N-(CH_2)_n-X \quad \rightarrow \quad W_1-A^2-NH(CH_2)_n-X$$

wherein A^2, n, W_1 and X are as defined above followed by deprotection of the W_1-group and coupling with the N-terminal amino acid, in a protected form, leading to the protected

peptide described in Method I. The synthesis to the final compounds is then continued according to Method I.

DETAILED DESCRIPTION OF THE INVENTION

The following description is illustrative of aspects of the invention.

EXPERIMENTAL PART

General Experimental Procedures.

The ^{1}H NMR and ^{13}C NMR measurements were performed on BRUKER AC-P 300, BRUKER 200 and BRUKER AM 500 spectrometers, the former operating at a ^{1}H frequency of 500.14 MHz and a ^{13}C freguency of 125.76 MHz and the latter at ^{1}H and ^{13}C freguency of 300.13 MHz and 75.46 MHz respectively.

The samples were 10-50 mg dissolved in 0.6 ml of either of the following solvents; $CDCl_3$ (isotopic purity > 99.8%, Dr. Glaser AG Basel), CD_3OD (isotopic purity > 99.95%, Dr. Glaser AG Basel) or D_2O (isotopic purity > 99.98%, Dr. Glaser AG Basel).

The ^{1}H and ^{13}C chemical shift values in $CDCl_3$ and CD_3OD are relative to tetramethylsilane as an external standard. The ^{1}H chemical shifts in D_2O are relative to the sodium salt of 3-(trimethylsilyl)-d_4-propanoic acid and the ^{13}C chemical shifts in D_2O are referenced relative to 1,4-dioxane (67.3 ppm), both as external standard. Calibrating with an external standard may in some cases cause minor shift differences compared to an internal standard, however, the difference in ^{1}H chemical shift is less than 0.02 ppm and in ^{13}C less than 0.1 ppm.

Thin-Layer Chromatography was carried out on commercial Merck Silicagel $60F_{254}$ coated glass or aluminium plates. Visualization was by a combination of UV-light, followed by spraying with a solution prepared by mixing 372 ml of EtOH(95%), 13.8 ml of concentrated H_2SO_4, 4.2 ml of concentrated acetic acid and 10.2 ml of p-methoxy benzaldehyde or phosphomolybdic acid reagent (5-10 w.t % in EtOH(95%)) and heating.

Flash chromatography was carried out on Merck Silicagel 60 (40-63 mm, 230-400 mesh) under pressure of N_2.

Freeze-drying was done on a Leybold-Heraeus, model Lyovac GT 2, apparatus.

Protection Procedures

Boc-(R)Cha-OH

To a solution of H-(R)Cha-OH, 21.55 g (125.8 mmol), in 130 ml 1 M NaOH and 65 ml THF was added 30 g (137.5 mmol) of $(Boc)_2O$ and the mixture was stirred for 4.5 h at room temperature. The THF was evaporated and an additional 150 ml of water was added. The alkaline aqueous phase was washed twice with EtOAc, then acidified with 2 M $KHSO_4$ and extracted with 3 x 150 ml of EtOAc. The combined organic phase was washed with water, brine and dried (Na_2SO_4). Evaporation of the solvent afforded 30.9 g (90.5 %) of the title compound as a white solid.

Preparation of Starting Materials

Boc-(R)Cha-OSu

Boc-(R)Cha-OH (1 eq.), HOSu (1.1 eq) and DCC or CME-CDI (1.1 eq) were dissolved in acetonitrile (about 2.5 ml/mmol acid) and stirred at room temperature over night. The precipitate

formed during the reaction was filtered off, the solvent evaporated and the product dried in vacuo. (When CME-CDI was used in the reaction the residue, after evaporation of the CH_3CN, was dissolved in EtOAc and the organic phase washed with water and dried. Evaporation of the solvent gave the title compound).

^1H-NMR (500 MHz, $CDCl_3$, 2 rotamers ca: 1:1 ratio) δ 0.85-1.1 (m, 2H), 1.1-1.48 (m, 4H), 1.5-1.98 (m, 16H; thereof 1.55 (bs, 9H)), 2.82 (bs, 4H), 4.72 (bs, 1H, major rotamer), 4.85 (bs, 1H, minor).

Boc-(R)Cha-Phe-OH

To a stirred mixture of 6.61 g (40 mmol) H-Phe-OH and 1.4 g of NaOH (35 mmol) in 60 ml DMF/H_2O (1/1) at + 5 °C was added 3.68 g (10 mmol) Boc-(R)Cha-OSu and the mixture was allowed to reach room temperature. After 3 hours the solvent was evaporated and the residue was dissolved in 150 ml of water. The basic water phase was washed with 2 x 50 ml EtOAc, acidified with 1 M $KHSO_4$ and extracted with 2 x 100 mL EtOAc. The combined organic phase was washed with 2 x 50 mL water and dried ($MgSO_4$). Filtration and evaporation of the solvent gave 2.86 g (68%) of the title compound.

Boc-(R)Cha-Phe-OSu

To a stirred solution of 2.81 g Boc-(R)Cha-Phe-OH (6.71 mmol) and 850 mg HOSu (7.38 mmol) in 30 mL of CH_3CN was added 3.13 g CME-CDI (7.38 mmol) and the reaction was left at room temperature for 15 hours. The precipitate formed during the reaction was filtered off, the solvent evaporated and the reasidue was dissolved in 150 mL EtOAc. The organic phase was washed with 1 x 20 mL water, 1 x 20 mL Na_2CO_3(aq), 2 x 20 mL water, 1 x 20 mL brine and dried ($MgSO_4$). Filtration followed by evaporation of the solvent gave 2.44 g (70%) of the title compound which was used without further purification.

Boc-Nag(Z)

(i) N-Bensyloxycarbonyl-O-methyl isourea

To a stirred solution of concentrated aqueous NaOH (2.8 L, 50% w/w, 19.1 M, 53 mol) and water (32 L) at 18° C was added in two portions O-methylisourea hemisulphate (1.7 kg, 94%, 13.0 mol) and 0-methylisourea hydrogensulphate (1.57 kg, 99%, 9.0 mol). The reaction mixture was cooled to 3-5° C. Benzyl chloroformiate (3.88 kg, 92%, 20.9 mol) was added over a 20 minutes period under cooling and vigorous stirring. The reaction temperature went from 3 to 8° C during the addition of Z-Cl. The addition funnel was rinsed with 5 litres of water which was added to the reactor. The reaction mixture was stirred at 0-3° C for 18 h, filtered and the crystals was washed with cooled (3° C) water (10 L). Vacuum drying (25° C, 10-20 mbar) for 48 h gave 3.87 kg (89%) of the title compound as a white crystalline powder.

(ii) Boc-Nag(Z)

To a stirred solution Boc-NH-$(CH_2)_3$-NH_2 x HCl (prepared according to Mattingly P.G., Synthesis, 367 (1990)) (3.9 kg, 18.5 mol) in iso-propanol (24 kg) at 60-70° C was added in portions over a 30 minutes period $KHCO_3$ (4.2 kg, 42 mol). A slow evolution of CO_2 (g) occurs. The mixture was stirred for another 30 minutes followed by addition in portions over a 30 minutes period N-bensyloxycarbonyl-O-methyl isourea (3.74 kg, 18.0 mol). The reaction mixture was stirred at 65-70° C for 16 h, cooled to 20° C and filtered. The precipitate was washed with iso-propanol (10 + 5 L). The combined filtrates was concentrated at reduced pressure keeping the heating mantle not warmer than 65-70° C. When approximately 45 litres was distilled off EtOAc (90 L) was added. The reaction mixture was cooled to 20-25° C, washed with water (10 and 5 L) and brine (5 L), and dried with Na_2SO_4 (2 kg). After stirring the rection mixture was filtered and the filter cake

was washed with EtOAc (11 and 7 L). The combined filtates were concentrated at reduced pressure keeping the heating mantle not warmer than 40-50° C. When approximately 90 litres of EtOAc was distilled off, toluene (25 L) was added and the evaporation continued. After collection of approximately another 18 litres of destillate, toulene (20 L) was added under vigorous stirring and the resulting mixture was cooled to -1 to 0° C and gently stirred over night (17 h). The crystal slurry was filtered and the product was washed with cooled toluene (10 and 5 L). Vacuum drying (10-20 mbar, 40° C) for 24 h gave 4.83 kg (13.8 mol, 76%) of Boc-Nag(Z).

^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.41 (s, 9H), 1.6-1.7 (m, 2H), 3.0-3.3 (m, 4H), 4.8-5.0 (bs, 1H), 5.10 (s, 2H), 7.2-7.4 (m, 5H).

Boc-Agm(Z)

(i) Boc-Agm

To a slurry of 14.95 g (65.5 mmol, 1 eq.) of agmatine sulphate (Aldrich), 13.7 ml of Et_3N (98.25 mmol, 1.5 eq.), 165 ml of H_2O and 165 ml of THF was added 21.5 g (98.25 mmol, 1.5 eq.) of $(Boc)_2O$ during 5 minutes at room temperature. The mixture was stirred vigorously over night, evaporated to dryness and the residue was washed with 2x100 ml of Et_2O to give Boc-Agm as a white powder which was used without further purification in the next step.

(ii) Boc-Agm(Z)

To a cold (+5°C) slurry of the crude Boc-Agm from the previous step (ca: 65.5 mmol) in 180 ml of 4N NaOH and 165 ml of THF was added 24 ml (169 mmol, 2.5 eq) of benzyl chloroformate during 10 minutes. After stirring at room temperature for 4 h methanol (150 ml) was added and the stirring was continued for an additional 20 h at room

temperature. The organic solvent was evaporated and 200 ml of H_2O was added to the residue. The basic water phase was extracted with 1x300 ml and 2x200 ml of EtOAc. The combined organic phases was washed with H_2O (2x100ml), brine (1x100 ml) and dried ($MgSO_4$). Evaporation of the solvent and flash chromathography (CH_2Cl_2/MeOH, a stepwise gradient of 97/3, 95/5 and 9/1 was used) gave 14.63 g (58%) of pure Boc-Agm(Z) as a white powder.

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.35-1.40 (m, 2H), 1.45 (s, 9H), 1.5-1.6 (m, 2H), 3.0-3.2 (m, 4H), 4.65 (bs, 1H), 5.1 (s, 2H), 7.25-7.40 (m, 5H).

^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 25.44, 27.36, 28.21, 65.83, 79.15, 127.47, 127.66, 128.14, 137.29, 156.47, 161.48, 163.30.

H-(R)Cha-Phe-Nag(Z)

(i) Boc-(R)Cha-Phe-OH

Boc-(R)Cha-OH was dissolved in acetonitrile (200 mL), N-hydroxisuccinimide (9.9 g, 81 mmol) was added. Dicyclohexylcarbodiimide (17.8 g, 81 mmol) was then added slowly and the reaction mixture was stirred overnight at room temperature. The precipitate was filtered off and the Boc-(R)Cha-OSu containing solution was evaporated. Phe-OH (48.7 g, 195 mmol), sodiumhydroxide (10.3 g, 258 mmol), water (270 mL) and finally dimethylformamide (70 mL) were added to a reaction vessel while stirring. Boc-(R)Cha-OSu was dissolved in dimethylformamide (200 mL) and added slowly to the reaction vessel while maintaining the reaction temperature below 5°C. After 3 h the solution was evaporated, the residue dissolved in water (1000 mL) and extracted with ethylacetate (2 x 300 mL). The aqueous phase was acidified with potassium hydrogensulfate (1M) to pH 3 and extracted with ethylacetate

(2 x 700 mL). The pooled organic layer was washed with water (2 x 300 mL) and dried over magnesiumsulfate. After filtration and evaporation the title product, Boc-(R)Cha-Phe-OH (21 g, 50 mmol) was isolated in 67% yield.

(ii) Boc-(R)Cha-Phe-Nag(Z)

Boc-(R)Cha-Phe-OH (20.8 g, 49.7 mmol) was dissolved in acetonitrile (350 mL). The vessel was cooled and 4-dimethylaminopyridine (12.1 g, 99.4 mmol) was added while maintaining a reaction temperature at 2°C. Nag(Z) (12.4 g, 49.7 mmol) was added resulting in a white slurry. Finally 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (8.6 g, 52 mmol) was added slowly over a 10 minute period. The solution was allowed to reach room temperature and stirred overnight. The solution was evaporated and the residue was dissolved in ethylacetate (400 mL) and water (150 mL). The organic layer is washed with potassium hydrogensulfate (1M, 250 mL), sodium carbonate (1M, 3 x 250 mL), water (200 mL) and finally brine (200 mL). The collected organic layer was evaporated and the title compound,
Boc(R)Cha-Phe-Nag(Z) (24.9 g, 38 mmol), was isolated in 77% yield.

(iii) H_2N-(R)Cha-Phe-Nag(Z)

Boc-(R)Cha-Phe-Nag(Z) (10 g, 15.4 mmol) was dissolved in ethylacetate (50 mL). The reaction vessel was cooled with ice-water bath to 4°C and HCl (46 mL, 152 mmol, 3.3 M in ethylacetate) was then added. The ice container was removed and the solution was allowed to reach room temperatur. After 1.5 h all starting material was consumed. The solvent was decanted from the HCl-salt of the deprotected peptide and the crude product was dissolved in water (50 mL) and extracted with ethylacetate(50 mL). The collected aqueous phase was neutralized by addition of potassium carbonate (4.3 g, 30.8 mmol) and then it extracted with dichloromethane (150 mL).

The collected organic phase was evaporated and H_2N-(R)Cha-Phe-Nag(Z) (3 g, 5.5 mmol), was isolated after purification by chromatography on silicagel (230-400 mesh) eluting with CH_2Cl_2:MeOH:NH_4OH (100:4:1 to 100:15:1) in 36% yield.
^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 7.82 (d, 1H), 7.4-7.1 (m, 10H), 5.09 (s, 2H), 4.42 (q, 1H), 3.4-2.9 (m, 7H), 1.8-0.7 (m, 15H).

Working Examples

Example 1

H-(R)Cha-Phe-Agm x 2 TFA

(i) Boc-(R)Cha-Phe-Agm(Z)

A solution of 729 mg (2 mmol) Boc-Agm(Z) in 15 mL TFA/CH_2Cl_2 (1:4) was stirred at room temperature for about 2 h. The solvent was evaporated and the product was dissolved together with 1.03 g (2 mmol) of Boc-(R)Cha-Phe-OSu in 10 mL DMF , the pH was adjusted with NMM to about 9 and the mixture was stirred at room temperature for 5 days. The solvent was evaporated in vacuo and the residue was dissolved in 200 mL EtOAc. The organic phase was washed with 2 x 10 mL of water, 1 M $KHSO_4$, 1 M NaOH, water and dried ($MgSO_4$). Evaporation of the solvent followed by flash chromatography (70 g SiO_2) using a stepwise gradient of 100 mL CH_2Cl_2/MeOH (95/5) followed by 250 mL CH_2Cl_2/MeOH (9/1) gave 1.09 g (96 %) of the title compound.

^1H-NMR (500 MHz, $CDCl_3$, mixture of two rotamers): major rotamer: δ 0.7-0.9 (m, 2H), 1.0-1.8 (m, 25H; thereof 1.39 (s, 9H)), 2.9-3.25 (m, 6 H), 4.02 (m, 1H), 4.71 (q, 1 H), 5.05 (s, 2 H), 7.1-7.4 (m, 10H).

^{13}C-NMR (125 MHz, D_2O): carbonyl and guanidine carbons: δ 161.7, 163.6, 172.0, 172.7 and 174.9.

(ii) H-(R)Cha-Phe-Agm x 2 TFA

A solution of 100 mg (0.15 mmol) Boc-(R)Cha-Phe-Agm(Z) in 10 mL CH_2Cl_2/TFA (4/1) was stirred at room temperature for 2 h 45 min after which the solvent was evaporated. The residue was dissolved in 9 ml EtOH/H_2O (8/1) and hydrogenated over 40 mg 5 % Pd/C at athmospheric pressure for 3 h. The catalyst was filtered off the solvent evaporated and the residue was dissolved in water and freeze dried to give 93 mg (94 %) of the title compound as a white powder.

^{1}H-NMR (500 MHz, D_2O, mixture of two rotamers): major rotamer: δ 0.65-1.75 (m, 17H), 2.86-3.23 (m, 6H), 3.96 (t, 1H), 4.59 (dd, 1H), 7.15-7.4 (m, 5H).

^{13}C-NMR (125 MHz, D_2O): guanidine δ 157.3; carbonyl carbons: δ 171.0 and 173.1.

Example 2

HOOC-CH_2-(R)Cha-Phe-Agm x 2 TFA

(i) H-(R)Cha-Phe-Agm(Z)

A solution of 0.99 g (1.49 mmol) Boc-(R)Cha-Phe-Agm(Z) in 30 mL CH_2Cl_2/TFA (4/1) was stirred at room temperature for 3 h after which the solvent was evaporated and the residue dissolved in 100 mL CH_2Cl_2. The organic phase was washed with 1 x 30 mL 5 M NaOH, 2 x 30 mL water and dried ($MgSO_4$). Filtration and evaporation of the solvent gave 825 mg (98 %) of the title compound as a white powder.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.75-1.0 (m, 2H), 1.05-1.75 (m, 15H), 2.93-3.34 (m, 7H), 4.56 (q, 1H), 7.13-7.39 (m, 10H).

^{13}C-NMR (75 MHz, $CDCL_3$): carbonyl and guanidine carbons: δ 161.8, 163.8, 171.8 and 176.5.

(ii) BnOOC-CH_2-(R)Cha-Phe-Agm(Z)

A mixture of 282 mg (0.5 mmol) H-(R)Cha-Phe-Agm(Z), 173 mg (1.25 mmol) K_2CO_3 and 137.5 mg (0.6 mmol) BnOOC-CH_2-Br in 16 mL CH_3CN/DMF (15/1) was heated to 50 °C for 4 h and 15 minutes after which the solvent evaporated and the residue dissolved in 70 mL EtOAc. The organic phase was washed with 4 x 10 mL water, 10 mL Brine and dried ($MgSO_4$). Evaporation of the solvent followed by flash chromatography (37 g SiO_2) using CH_2Cl_2/MeOH(NH_3-saturated) (95/5) as eluent afforded 230 mg (64 %) of the desired compound.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.7-0.95 (m, 2H), 1.05-1.75 (m, 15H), 2.84-3.25 (m, 8H), 4.56-4.68 (m, 1H), 4.95 (s, 2H), 5.12 (s, 2H), 7.1-7.45 (m, 15H).

^{13}C-NMR (75 MHz, $CDCL_3$): carbonyl and guanidine carbons: δ 161.7, 163,6, 171.56, 171.61 and 175.1.

(iii) HOOC-CH_2-(R)Cha-Phe-Agm x 2 TFA

To a solution of 230 mg (0.323 mmol) BnOOC-CH_2-(R)Cha-Phe-Agm(Z) in 18 mL EtOH/H_2O (5/1) was added a small amount (15 drops) of TFA and the mixture was hydrogenated over 70 mg 5 % Pd/C at athmospheric pressure for 6 h. The catalyst was filtered off, the solvent was evaporated and the residue was dissolved in water and freeze dried to afford 223 mg (96%) off the title compound as a white powder.

^{1}H-NMR (300 MHz, CD_3OD): δ 0.65-1.85 (m, 17H), 2.8-3.0 (m, 1H), 3.0-3.3 (m, 5H), 3.78 (bs, 2H), 4.0 (bs, 1H), 4.62 (m, 1H), 7.1-7.4 (m, 5H).

^{13}C-NMR (75 MHz, CD_3OD): guanidine: δ 158.6; carbonyl carbons: δ 168.9, 169.6 and 173.4.

Example 3

H-(R)Cha-Phe-Nag x 2 TFA

(i) Boc-(R)Cha-Phe-Nag(Z)

Prepared in the same way as described for Boc-(R)Cha-Phe-Agm(Z) in Example 1 (i) from Boc-(R)Cha-Phe-OSu (2 mmol) and Boc-Nag(Z) (2 mmol).Yield = 1.02 g (78 %).

(ii) H-(R)Cha-Phe-Nag x 2 TFA

A solution of 100 mg (0.15 mmol) Boc-(R)Cha-Phe-Nag(Z) in 10 mL CH_2Cl_2/TFA (4/1) was stirred at room temperature for 3 h 35 min after which the solvent was evaporated. The residue was dissolved in 9 ml EtOH/H_2O (8/1) and hydrogenated over 40 mg 5 % Pd/C at athmospheric pressure for 3 h. The catalyst was filtered off the solvent evaporated and the residue was dissolved in water and freeze dried to give 97 mg (98 %) of the title compound as a white powder.

^{1}H-NMR (500 MHz, D_2O, mixture of two rotamers): major rotamer: δ 0.75-1.85 (m, 15H), 2.9-3.45 (m, 6H), 4.05 (t, 1H), 4.6-4.8 (m, 1H; partially hidden by the H-O-D signal), 7.3-7.6 (m, 5H).

^{13}C-NMR (75 MHz, D_2O): guanidine δ 157.6; carbonyl carbons:171.3 and 173.5.

Example 4

HOOC-CH_2-(R)Cha-Phe-Nag x 2 TFA

(i) H-(R)Cha-Phe-Nag(Z)

Prepared in the same way as described for H-(R)Cha-Phe-Agm(Z) in Example 2 (i) from Boc-(R)Cha-Phe-Nag(Z). Yield 90 %.

^{13}C-NMR (75 MHz, $CDCl_3$): δ 26.0, 26.2, 26.4, 29.5, 32.2, 34.0, 34.2, 36.7, 37.7, 38.3, 42.6, 52.7, 55.1, 66.3, 126.9, 127.7, 127.9, 128.3, 128.6, 129.1, 136.7, 137.5, 161.8, 163.7, 171.5 and 176.6.

(ii) BnOOC-CH_2-(R)Cha-Phe-Nag(Z)

A mixture of 275 mg (0.5 mmol) H-(R)Cha-Phe-Nag(Z), 173 mg (1.25 mmol) K_2CO_3 and 137.5 mg (0.6 mmol) BnOOC-CH_2-Br in 15 mL CH_3CN was heated to 50 °C for 3 h and 50 minutes after which the solvent evaporated and the residue dissolved in 70 mL EtOAc. The organic phase was washed with 4 x 10 mL water, 10 mL Brine and dried ($MgSO_4$). Evaporation of the solvent followed by flash chromatography (37 g SiO_2) using CH_2Cl_2/MeOH(NH_3-saturated) (95/5) as eluent afforded 209 mg (60 %) of the desired compound.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.72-0.93 (m, 2H), 1.0-1.72 (m, 13H), 2.83-3.25 (m, 9H), 4.54 (q, 1H), 5.09 (s, 2H), 5.11 (s, 2H), 7.05-7.4 (m, 15H), 7.59 (d, 1h; NH).

^{13}C-NMR (75 MHz, $CDCl_3$): carbonyl and guanidine carbons: δ 161.8, 163.6, 171.3, 171.6 and 175.2.

(iii) HOOC-CH_2-(R)Cha-Phe-Nag x 2 TFA

To a solution of 209 mg (0.3 mmol) BnOOC-CH_2-(R)Cha-Phe-Nag(Z) in 18 mL EtOH/H_2O (5/1) was added a small amount (15 drops) of TFA and the mixture was hydrogenated over 70 mg 5 % Pd/C at athmospheric pressure for 4 h. The catalyst was filtered off, the solvent was evaporated and the residue was dissolved in water and freeze dried to afford 190 mg (90%) off the title compound as a white powder.

^{1}H-NMR (300 MHz, CD_3OD): δ 0.6-1.38 (m, 6H), 1.4-1.9 (m, 9H), 2.9-3.4 (m, 6H), 3.9 (bs, 2H), 4.1 (bs, 1H), 4.7 (m, 1H; partially hidden by the H-O-D signal), 7.1-7.45 (m, 5H).

^{13}C-NMR (75 MHz, CD_3OD): guanidine: δ 157.5; carbonyl carbons: δ 169.3, 169.5 and 173.2.

Example 5

H-(R)Cha-Phe-Nag

H_2N-(R)Cha-Phe-Nag(Z) (300 mg, 0.55 mmol) was dissolved in ethanol (50 mL) and trifluoroaceticacid (56 μL, 0.73 mmol) was added. The mixture was sonicated and palladium on charcoal (5%, 50 mg) was charged before it was hydrogenated at 45 psi hydrogen pressure in a Parr shaking apparatus for 19 h. The suspension was filtered through celite and after the solvent was evaporated the title compound (0.13 g, 0.31 mmol) was isolated in 56% yield.
^{1}H NMR (200 MHz, d-HCl+d_2-H_2O) δ (ppm) 7.40-7.00 (m, 5H), 4.47 (t, 1H), 3.86 (t, 1H), 3.25-2.65 (m, 6H), 1.75-0.50 (m, 15H).
TSP-MS found (m/z)=417 (calc. for MH+($C_{22}H_{37}N_6O_2$)417).

Example 6

CH_3-CO-(R)Cha-Phe-Nag

(i) CH_3-CO-(R)Cha-Phe-Nag(Z)

H_2N-(R)Cha-Phe-Nag(Z) (500 mg, 0.91 mmol) was dissolved in acetonitrile (7.5 mL). Acetylchloride (107 mg, 1.36 mmol) dissolved in acetonitrile (1 mL) was then transferred to the reaction vessel. After 30 min. the acylated peptide precipitated as an HCl-salt. Diethylether (5 mL) was added 20 minutes later. The precipitate was filtered off and dried under vacuum at 35°C overnight and the dry product, CH_3-CO-(R)Cha-Phe-Nag(Z)xHCl (407 mg, 0.69 mmol) was isolated in 76% yield.

1H NMR (200 MHz, CDCl3) δ (ppm) 7.4-7.1 (m, 10H), 5.1 (q, 2H), 4.7 (m, 1H), 4.0 (m, 1H), 3.5-2.9 (m, 6H), 2.0-0.6 (m, 18H). TSP-MS found (m/z)=593 (calc. for $MH^+(C_{32}H_{45}N_6O_5)$593).

(ii) CH_3-CO-(R)Cha-Phe-Nag

CH_3-CO-(R)Cha-Phe-Nag(Z) (400 mg, 0.68 mmol) was dissolved in ethanol (60 mL) and palladium on charcoal (5%, 80 mg) was added. The mixture was hydrogenated at 45 psi hydrogen pressure in a Parr shaking apparatus for 20 h. the suspension was filtered through celite and after the solvent was evaporated a crude mixture (300 mg) was collected. The crude product (150 mg) was purified by reveresed phase chromatography (C8-gel) eluting with MeCN:NH4OAc (0.1M) (40:60) and the product (100 mg, 0.22 mmol) in 64% yield.

^1H NMR (200 MHz, d_4-CH_3OH) δ (ppm) 7.25-6.85 (m, 5H), 4.44 (dd, 1H), 4-02 (t, 1H), 3.30-2.90 (m, 5H), 2.72 (dd, 1H), 2.0-0.5 (m, 18H). TSP-MS found (m/z)459 (calc. for $MH^+(C_{24}H_{39}N_6O_3)$459).

Example 7

CH_3CH_2-(R)Cha-Phe-Nag

(i) CH_3CH_2-(R)Cha-Phe-Nag(Z)

H_2N-(R)Cha-Phe-Nag(Z) (500 mg, 0.91 mmol), p-toluenesulphonic acid (173 mg, 0.91 mmol) and methanol (7.5 mL) were added to

a reaction vessel which was cooled with ice. Acetaldehyde (51 μL, 0.91 mmol) was added and finally after another 30 min., sodium cyanoborohydride (86 mg, 1.36 mmol) was added. The mixture was stirred at room temperature for four days and then evaporated. The crude product was purified by chromatography on silicagel (230-400 mesh) eluting with $MeCl_2$: MeOH: NH_4OH (90:10:1) yielding CH_3CH_2-(R)Cha-Phe-Nag(Z) (150 mg, 0.26 mmol) in 29% yield.
^1H NMR (200 MHz, d_4-MeOH) δ (ppm) 7.39-7.28 (m, 10H), 5.11 (s, 2H), 4.63 (t, 1H, 3.2-2.9 (m), 2.42 (m, 2H), 1.8-0.8 (m, 18H).

(ii) CH_3CH_2-(R)Cha-Phe-Nag(Z) 150 mg, 0.26 mmol) was dissolved in EtOH 840 mL) and acetic acid (1 mL). Palladium on charcoal (5%, 51 mg) was charged before it was hydrogenated at 45 psi hydrogen pressure in a Parr shaking apparatur for 2 days. The mixture was filtered and the filter cake was washed with MeOH/AcOH (2:1, 40 mL). The title compound (32 mg, 0.072 mmol) was isolated in 28% yield by chromatography on silicagen (230-400 mesh) eluting with heptane: EtOAc: TEA (30:70:1).
^1H NMR (200 MHz, d_4-MeOH) δ (ppm) 7.50-7.10 (m, 5H), 4.62 (q, 1H), 3.76-3.67 (m, 1H), 3.65-3.56 (m, 1H), 3.51 (t, 1H), 3.35-2.95 (m, 6H), 2.70 (q, 2H), 2.0-0.5 (m, 18H).

Example 8

HOOC-CO-(R)Cha-Phe-Nag

(i) HOOC-CO-(R)Cha-Phe-Nag(Z)

H_2N-(R)Cha-Phe-Nag(Z) (500 mg, 0.91 mmol) was dispersed in acetonitrile (5 mL). Methyloxalylchloride (104 μL, 1.14 mmol) was added to the slurry. After 60 minutes the starting material was consumed, confirmed by HPLC, and the clear solution was evaporated.

The crude methylester was hydrolyzed by dissolving the residue in tetrahydrofuran (4 mL) and adding LiOH (115 mg, 2.73 mmol) dissolved in water (2 mL). After 90 min. more LiOH (70 mg, 1.7 mmol) was added and 30 minutes later water (10 mL) was added and the insoluble material were dissolved. After evaporation the dry uncolored powder was slurried in water (10 mL) containing ammonium chloride (150 mg). The mixture was stirred for 30 min. and then the precipitate was filtered and washed with two portions of water.
^{1}H NMR (200 MHz, d_6-DMSO) δ (ppm) 8.7 (d, 1H), 8.2-7.6 (m), 7.31-7.22 (m, 10H), 4.94 (s, 2H), 4.32 (m, 1H), 4.00 (m, 1H), 3.3-2.6 (m, 6H), 1.7-0.6 (m, 15H). TSP-MS found (m/z) 623 (calc. for $MH^+(C_{32}H_{43}N_6O_7)$623).

(ii) HOOC-CO-(R)Cha-Phe-Nag

HOOC-CO-(R)Cha-Phe-Nag(Z) (210 mg, 0.34 mmol) was dispersed in tetrahydrofuran (25 mL) and acitic acid (20 mL) was added. Palladium on charcoal (5%, 30 mg) was charged before it was hydrogenated at 45 psi hydrogen pressure in a Parr shaking apparatus for 25 h. The suspension was filtered through celite and the filter cake was washed with tetrahydrofuran and after the solvent was evaporated the crude product (257 mg) was collected. After azeotropic evaporation with three portions of toluene (tot; 50 mL) and overnight drying under vacuum the product (140 mg, 0.29 mmol) was isolated in 85% yield.
^{1}H NMR (200 MHz, D_4-MeOH) δ (ppm) 7.19 (m, 5H), 4.54 (dd, 1H), 4.00 (t, 1H), 4.00 (t, 1H), 3.50-2.90 (m, 5H), 2.70 (t, 1H), 1.90-0.60 (m, 15H). TSP-MS found (m/z) 489 (calc. for $MH^+(C_{24}H_{36}N_6O_5)$489).

Pharmaceutical preparations

A. The compounds according to the invention can be formulated in solid dosage forms for oral administration or for topical administration to the intestines.

Example A1

Plain tablet

Kininogenase inhibitor	10 mg/tablet
Lactose anhydrous	250 mg/tablet
Microcrystalline cellulose	60 mg/tablet
Magnesium stearate	6 mg/tablet

The active constituent is mixed with lactose and microcrystalline cellulose and magnesium stearate is admixed and tablets are compressed from the mixture.

Example A2

Coated tablet

Kininogenase inhibitor	100 mg/tablet
Lactose	300 mg/tablet
Polyvinylpyrrolidone	40 mg/tablet
Magnesium stearate	8 mg/tablet
Hydroxypropylmethylcellulose	8 mg/tablet
Polyetheleneglycol	1 mg/tablet
Talc	1 mg/tablet
Titandioxid	1 mg/tablet

The active constituent is mixed with lactose and granulated with polyvinylpyrrolidone in water. After drying and milling magnesium stearate is admixed and tablets are compressed. The tablets are coated with a solution of hydroxypropylmethylcellulose, polyethyleneglycol, talc and titandioxide in water.

Example A3

Gastro-resistant tablet

Kininogenase inhibitor	10 mg/tablet
Lactose	200 mg/tablet
Polyvinylpyrrolidone	40 mg/tablet

Microcrystalline cellulose	50 mg/tablet
Magnesium stearate	8 mg/tablet
Eudragit L	10 mg/tablet
Dibutylphtalate	1 mg/tablet
Talc	2 mg/tablet

The active constituent is mixed with lactose and granulated with polyvinylpyrrolidone in water. After drying and milling microcrystalline cellulose and magnesium stearate is admixed and tablets are compressed. The tablets are coated with a solution of Eudragit L, dibutylphtalate and talc in isopropanol/aceton.

Example A4

Gastro-resistant extended release granules for the small intestine

Kininogenase inhibitor	100 mg/g
Lactose	448 mg/g
Microcrystalline cellulose	200 mg/g
Hydroxypropyl cellulose	50 mg/g
Ethylcellulose	20 mg/g
Acetyltributylcitrate	2 mg/g
Eudragit L30D	50 mg/g
Triethylcitrate	5 mg/g
Talc	25 mg/g

The active constituent is mixed with lactose and microcrystalline cellulose and granulated with hydroxypropyl cellulose in water. The granulation is extruded, spheronized and dried. The granules are first coated with ethylcellulose dispersion with acetyltributylcitrate and then with Eudragit L30D dispersion with triethylcitrate and talc. The granules are filled in gelatin capsules each containing 10 mg of active constituent.

Example A5

Gastro-resistant extended release granules for the colon

Kininogenase inhibitor	200 mg/g
Lactose	400 mg/g
Microcrystalline cellulose	200 mg/g
Hydroxypropyl cellulose	50 mg/g
Eudragit NE30D	50 mg/g
Eudragit S100	50 mg/g
Talc	50 mg/g

The active constituent is mixed with lactose and microcrystalline cellulose and granulated with hydroxypropyl cellulose in water. The granulation is extruded, spheronized and dried. The granules are coated with a dispersion of Eudragit NE30D, Eudragit S100 and talc in water. The granules are filled in gelatin capsules each containing 100 mg of active constituent.

B. The compounds according to the invention can be formulated in pressurized aerosols or in dry powder inhalers for oral or nasal inhalation. The kininogenase inhibitor is micronized to a particle size suitable for inhalation therapy (mass median diameter < 4μm).

For pressurized aerosols the micronized substance is suspended in a liquid propellant mixture and filled into a container which is sealed with a metering valve. Alternatively, the kininogenase inhibitor can be dissolved in the liquid propellant mixture with the aid of ethanol.

The propellants used may by chlorofluorocarbons (CFCs) or hydrofluoroalkanes (HFAs) of different formulae. The most frequent used CFCs are trichloromonofluoromethane (propellant 11) and dichlorodifluoromethane (propellant 12) and dichlorotetrafluoroethane (propellant 114). The most frequent

used HFAs are tetrafluoromethane (propellant 134a) and heptafluoropropane (propellant 227).

Low concentrations of surfactant such as sorbitan trioleate, lecithin, oleic acid or other suitable substance may be used to improve the physical stability. Etanol may be used as surfactant or as a medium to increase the solubility of active substance in the propellant mixture.

Example B1	per cent (w/w)
Kininogenase inhibitor	0.5
Trichloromonofluoromethane (propellant 11)	15
Dichlorodifluoromethane (propellant 12)	84
Sorbitan trioleate	0.5

Example B2	per cent (w/w)
Kininogenase inhibitor	0.5
Trichloromonofluoromethane (propellant 11)	25
Dichlorodifluoromethane (propellant 12)	74.48
Oleic acid	0.02

Example B3	per cent (w/w)
Kininogenase inhibitor	0.2
Trichloromonofluoromethane (propellant 11)	15
Dichlorodifluorometane	64.78
Ethanol	20
Oleic acid	0.02

Example B4	per cent (w/w)
Kininogenase inhibitor	0.4
Tetrafluoroethane (propellant 134a)	59.58

Heptafluoropropane (propellant 227)	20
Ethanol	20
Oleic acid	0.02

Example B5	per cent (w/w)
Kininogenase inhibitor	1.0
Heptafluoropropane (propellant 227)	93.5
Ethanol	5
Sorbitan trioleate	0.5

In a dry powder inhaler the micronized kininogenase inhibitor may be used alone or mixed with a carrier substance such as lactose, mannitol or glucose. Another possibility is to process the micronized powder into spheres which break up during the dosing procedure. This powder or spheronized powder is filled into the drug reservoir in a singledose or multidose inhaler, e.g. the latter being Turbuhaler®. A dosing unit meters the desired dose which is inhaled by the patient.

Example B6

The kininogenase inhibitor is micronized in a jet mill to a particle size suitable for inhalation (mass diameter < 4μm). 100 mg of the micronized powder is filled into a powder multidose inhaler (Turbuhaler®). The inhaler is equipped with a dosing unit which delivers a dose of 1 mg.

Example B7

The kininogenase inhibitor is micronized in a jet mill to a particle size suitable for inhalation (mass diameter < 4μm). 150 mg of the micronized powder is filled into a powder multidose inhaler (Turbuhaler®). The inhaler is equipped with a dosing unit which delivers a dose of 0.5 mg.

ABBREVIATIONS

Ac =	Acetyl
Agm =	Agmatine
Agm(Z) =	ω-N-benzyloxycarbonyl agmatine
Boc =	tertiary butoxy carbonyl
Brine =	saturated water/NaCl solution
Bn =	benzyl
Cha =	(S)-β-cyclohexyl alanine
CME-CDI =	1-Cyclohexyl-3-(2-morpholino-ethyl)carbodiimide metho-p-toluenesulfonate
DCC =	dicyclohexyl carbodiimide
DMF =	dimethyl formamide
Et =	ethyl
EtOAc =	ethyl acetate
HOSu =	N-hydroxysuccinimide
HPLC =	High Performance Liquid Chromatography
LiOH =	Lithium hydroxide
Me =	methyl
Nag =	noragmatine
Nag(Z) =	ω-N-benzyloxycarbonyl-noragmatine
Nal	(S)-naphthylalanine
NMM =	N-methyl morpholine
Ph =	phenyl
Phe =	(S)-phenylalanine
Pro =	(S)-proline
Ser =	(S)-serine
TFA =	trifluoracetic acid
THF =	tetrahydrofuran
Z =	benzyloxycarbonyl

Prefixes n, s, i and t have their usual meanings: normal, iso, sec and tertiary. The stereochemistry for the amino acids is by default (S) if not otherwise stated.

CLAIMS

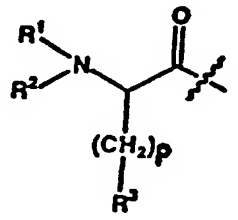
1. A compound of the general formula

$A^1-A^2-NH-(CH_2)_n-NH-C(NH)-NH_2$ Formula I

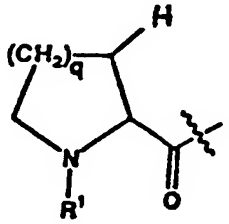
wherein:

n is an integer 2, 3, 4, 5, or 6; preferably 3 or 4;

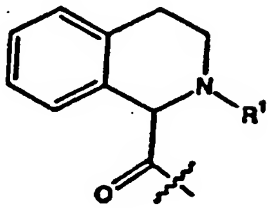
A^1 represents a structural fragment of Formulae IIa, IIb, IIc, IId or IIe;



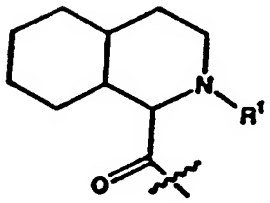
IIa



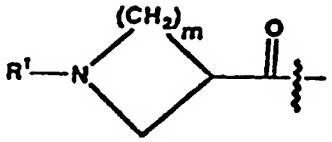
IIb



IIc



IId



IIe

wherein:

p is an integer 0,1 or 2;

m is an integer 1, 2, 3, or 4, preferably 2;

q is an integer 0-2, preferably 1;

R^1 represents H, an alkyl group having 1 to 4 carbon atoms, a hydroxyalkyl group having 2-3 carbon atoms or $R^{11}OOC$-alkyl-, where the alkyl group has 1 to 4 carbon atoms and R^{11} is H or an alkyl group having 1 to 4 carbon atoms, or

R^1 represents $R^{12}OOC$-1,4-phenyl-CH_2-, wherein R^{12} is H or an alkyl group having 1 to 4 carbon atoms, or

R^1 represents R^{13}-NH-CO-alkyl-, wherein the alkyl group has 1 to 4 carbon atoms and is possibly substituted alpha to the carbonyl with an alkyl group having 1 to 4 carbon atoms and where R^{13} is H or an alkyl group having 1 to 4 carbon atoms or $-CH_2COOR^{12}$, wherein R^{12} is as defined above, or

R^1 represents $R^{14}SO_2$-, Ph(4-$COOR^{12}$)-SO_2-, Ph(3-$COOR^{12}$)-SO_2-, or Ph(2-$COOR^{12}$)-SO_2-, wherein R^{12} is as defined above and R^{14} is an alkyl group having 1-4 carbon atoms, or

R^1 represents CO-R^{15}, wherein R^{15} is an alkyl group having 1-4 carbon atoms, or

R^1 represents CO-OR^{15}, wherein R^{15} is as defined above, or

R^1 represent CO-$(CH_2)_p$-$COOR^{12}$, wherein R^{12} and p are as defined above, or

R^1 represents -$CH_2PO(OR^{16})_2$, wherein R^{16} is, individually at each occurrence, H, methyl or ethyl;

R^2 represents H or an alkyl group having 1 to 4 carbon atoms or $R^{21}OOC$-alkyl-, wherein the alkyl group has 1 to 4 carbon atoms and is possibly substituted in the position which is alpha to the carbonyl group, and the alpha substituent is a group R^{22}-$(CH_2)_p$-, wherein p is as defined above and R^{22} is methyl, phenyl, OH, $COOR^{21}$, and R^{21} is H or an alkyl group having 1 to 4 carbon atoms;

R^3 represents an alkyl group having 1-4 carbon atoms, or

R^3 represents a cyclohexyl- or cyclopentyl group, or

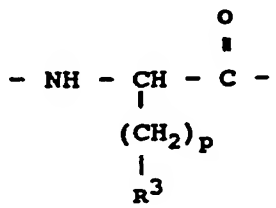
R^3 represents a phenyl group which may or may not be substituted with an alkyl group having 1 to 4 carbon atoms, or with a group OR^{21}, or

R^3 represents a 1-napthyl, 2-naphtyl, 4-pyridyl, 3-pyrrolidyl, or a 3-indolyl group which may or may not be substituted with OR^{21} and with p = 1; or

R^3 represent a cis- or trans-decalin group with p = 1; or

R^3 represents $Si(Me)_3$ or $CH(R^{31})_2$, wherein R^{31} is a cyclohexyl- or phenyl group;

A^2 represents a structural fragment



wherein R^3 and p are as defined above.

2. A compound according to claim 1 wherein A^1 represents IIa or IIb.

3. A compound according to claim 1 wherein A^1 represents IIa.

4. A compound according to claim 1 wherein R^3 is cyclohexyl, cyclopentyl, phenyl, substituted phenyl or other aryl systems and p is 1.

5. A compound according to claim 2 wherein R^3 is cyclohexyl, cyclopentyl, phenyl, substituted phenyl or other aryl systems and p is 1.

6. A compound according to claim 3 wherein R^3 is cyclohexyl, cyclopentyl, phenyl, substituted phenyl or other aryl systems and p is 1.

7. A compound according to one or more of the preceding claims wherein R^1 represents $R^{11}OOC$-alkyl-, wherein the alkyl group has 1 to 4 carbon atoms and R^{11} is H.

8. A compound according to one or more of the preceding claims wherein R^3 is cyclohexyl or substituted phenyl.

9. A compound according to one or more of the preceding claims wherein n is 3 or 4.

10. A compound according to one or more of the preceding claims wherein n is 4.

11. A compound according to one or more of the preceding claims wherein p is 1.

12. A compound according to one or more of the preceding claims wherein q is 1.

13. A compound according to one or more of the preceding claims having R-configuration on the amino acid fragment in the A^1 position.

14. A compound according to one or more of the preceding claims having S-konfiguration on the amino acid fragment in the A^2 position.

15. A compound selected from

H-(R)Cha-Phe-Agm
HOOC-CH_2-(R)Cha-Phe-Agm
H-(R)Cha-Phe-Nag
HOOC-CH_2-(R)Cha-Phe-Nag
CH_3-CO-(R)Cha-Phe-Nag
CH_3-CH_2-(R)Cha-Phe-Nag
HOOC-CO-(R)Cha-Phe-Nag

either as such or in the form of a physiologically acceptable salt and including stereoisomers.

16. The compound HOOC-CH_2-(R)Cha-Phe-Nag, either as such or in the form of a physiologically acceptable salt and including stereoisomers.

17. A process for preparing a compound according to claim 1, which process comprises coupling of a N-terminally protected dipeptide (W_1-A^1-A^2-OH) or amino acid (W_1-A^1-OH) when a N-terminally protected amino acid is used a second amino acid is added afterwards using standard methods, to a compound

$$H_2N-(CH_2)_n-X$$

wherein A^1, A^2 and n are as defined in Formula I, W_1 is an amino protecting group and X is an unprotected or protected guanidino group or a protected amino group, or a group transferable into an amino group, where the amino group is subsequently transferred into an unprotected or protected guanidino group, followed by removal of the protecting group(s) or deprotecting of the N-terminal nitrogen followed by alkylation of the N-terminal nitrogen and deprotection by known methods.

and if desired forming a physiologically acceptable salt, and in those cases where the reaction results in a mixture of stereoisomers, these are optionally separated by standard

chromatographic or re-crystallisation techniques, and if desired a single stereoisomer is isolated.

18. A process according to claim 17 which process comprises

a) Method I

Coupling of an N-terminally protected dipeptide, prepared by standard peptide coupling, with either a protected- or unprotected amino guanidine or a straight chain alkylamine carrying a protected or masked amino group at the terminal end of the alkyl chain, using standard peptide coupling, shown in the formula

$$W_1\text{-}\ A^1\text{-}A^2\text{-}OH \xrightarrow{\downarrow\ H_2N\text{-}(CH_2)_n\text{-}X} W_1\text{-}A^1\text{-}A^2\text{-}NH(CH_2)_n\text{-}X$$

wherein A^1, A^2 and n are as defined in Formula I , W_1 is a N-teminal amino protecting group such as tert-butyloxy carbonyl and benzyloxy carbonyl and X is $-NH-C(NH)-NH_2$, $-NH-C(NH)-NH-W_2$, $-N(W_2)-C(NH)-NH-W_2$, $-NH-C(NW_2)-NH-W_2$ or $-NH-W_2$, where W_2 is an amine protecting group such as tert-butyloxy carbonyl or benzyloxy carbonyl, or X is a masked amino group such as azide, giving the protected peptide, and further depending on the nature of the X- group used: Removal of the protecting group(s) (when X= $-NH-C(NH)-NH_2$, $-N(W_2)-C(NH)-NH-W_2$, $-NH-C(NW_2)-NH-W_2$ or $-NH-C(NH)-NH-W_2$), or a selective deprotection of the W_1- group (e.g when X= $-NH-C(NH)-NH-W_2$, $-N(W_2)-C(NH)-NH-W_2$, $-NH-C(NW_2)-NH-W_2$, W_2 in this case must be orthogonal to W_1) followed by alkylation of the N-terminal nitrogen and deprotection or a selective deprotection/ unmasking of the terminal alkylamino function (X= $NH-W_2$, W_2 in this case must be orthogonal to W_1 or X= a masked aminogroup, such as azide)

followed by a guanidation reaction, using standard methods, of the free amine and deprotection of the W_1-group, or

b) Method II

Coupling of a N-terminally protected amino acid, prepared by standard methods, with either a protected- or unprotected amino guanidine or a straight chain alkylamine carrying a protected or masked amino group at the terminal end of the alkyl chain, using standard peptide coupling, shown in the formula

$$W_1\text{-}A^2\text{-}OH \;\downarrow\; H_2N\text{-}(CH_2)_n\text{-}X \;\rightarrow\; W_1\text{-}A^2\text{-}NH(CH_2)_n\text{-}X$$

wherein A^2, n, W_1 and X are as defined above followed by deprotection of the W_1-group and coupling with the N-terminal amino acid, in a protected form, leading to the protected peptide described in Method I, whereafter the synthesis to the final compounds is continued according to Method I.

19. Use of a compound of the formula I

$$A^1\text{-}A^2\text{-}NH\text{-}(CH_2)_n\text{-}NH\text{-}C(NH)\text{-}NH_2$$

according to claim 1,

either as such or in the form of a salt, and as such or having the guanidino group either mono protected at the δ-nitrogen or diprotected at the δ-nitrogens or the γ, δ-nitrogens, as a starting material in synthesis of a serine protease inhibitor, and in particular in synthesis of a kininogenase inhibitor.

20. Use according to claim 19, wherein the serine protease inhibitor is a peptidic compound.

21. A compound according to claim 1 for use in therapy.

22. A compound according to claim 21 for use as an antiinflammatory agent.

23. A pharmaceutical preparation comprising an effective amount of a compound according to claims 1 in conjunction with one or more pharmaceutical carriers.

24. A pharmaceutical preparation according to claim 23 for use as an antiinflammatory agent.

25. Use of compound according to claim 1 as an active ingredient for manufacture of a pharmaceutical preparation for inhibition of serine proteases and in particular kininogenases in a human or animal organism.

26. A method for obtaining inhibition of serine proteases and in particular kininogenases in a human or animal organism in need of such inhibition, comprising administering to said organism an inhibitory effective amount of a compound according to claim 1.

27. A compound, a process, a pharmaceutical preparation, a use and a method as claimed in any of claims 1-26 and substantially as described.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 94/00534

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: C07K 5/06, C07K 5/02, A61K 37/64
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: A61K, C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, MEDLINE, EMBASE, CA, WPI, CLAIMS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BRAZILIAN J MED BIOL RES, Volume 20, 1987, L.A.F. FERREIRA et al, "Kallikrein isolated from commersial crystalline pepsin preparations" page 511 - page 520	1,2,4,5, 8-10,12-14, 19,20,25
A	GB, A, 2085444 (RICHTER GEDEON VEGYESZETI GYAR RT), 28 April 1982 (28.04.82)	1-25
A	WO, A1, 9204371 (FERRING PEPTIDE RESEARCH PARTNERSHIP KB), 19 March 1992 (19.03.92)	1-25

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"B" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
30 August 1994

Date of mailing of the international search report
08 -09- 1994

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 94/00534

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 26
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [X] Claims Nos.: 27
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claim 27, which concerns different kinds of categories in the same claim, is not searchable. See PCT Article 6.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/07/94

International application No.
PCT/SE 94/00534

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB-A- 2085444	28/04/82	AT-B- 384228	12/10/87
		AU-B- 535688	29/03/84
		AU-A- 6672581	22/04/82
		BE-A- 887224	27/07/81
		CA-A- 1158641	13/12/83
		DE-A- 3108810	19/05/82
		FR-A,B- 2491463	09/04/82
		JP-A- 57064653	19/04/82
		NL-A- 8100391	03/05/82
		SE-B,C- 452326	23/11/87
		SE-A- 8100302	08/04/82
		SU-A- 1178322	07/09/85
WO-A1- 9204371	19/03/92	AU-A- 8438791	30/03/92
		JP-T- 6501461	17/02/94